INFLUENCE OF WASH SOLUTIONS ON MECHANICAL AND SURFACE PROPERTIES OF 3D-PRINTED MATERIALS

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To assess the influence of 3 different wash solutions on the mechanical and surface properties of a 3D-printed glass-reinforced composite resin (3D-CR).

One bar (25 x 2 x 2 mm) and 2 types of discs (15 x 1 mm/15 x 3 mm) STL files were used to fabricate all samples by 3D printing with a commercially available 3D-CR. Three groups were created based on the wash solution used in an ultrasonic bath: InovaPrint Wash (group 1), ethanol 96% (group 2), and denatured ethyl alcohol 90° (group 3). The final set of samples included 30 bars and disks (15 for each thickness). To mimic the oral cavity, all samples were immersed in distilled water at 37±1°C for 30 days. The samples were subjected to a 3-bonding test to evaluate flexural strength (FS) and Young's modulus (E) (ISO 4049), water sorption (Wsp) and solubility (Wsl) tests (ISO 10477), Vickers hardness tests (VHN), and toothbrushing simulations (ISO 14569-2) to evaluate surface roughness (ISO 16610-21). The Kruskal-Wallis test and Bonferroni post-hoc were used to compare groups.

Significant difference emerged ($p < .0001$) between groups for FS, E, and VHN. For FS, post-hoc showed the difference between groups 1 *vs* 3 and 2 *vs* 3. Instead, for E, significance was found between groups 1 *vs* 2. For VHN, between groups 1 *vs* 2 and 2 *vs* 3. Differences between groups were observed in Wsp and Wsl at 21 and 28 days. In terms of surface roughness, the materials showed values above the clinical threshold before and after brushing.

The wash solutions affected the mechanical and surface properties of 3D-CR, but all values were below the thresholds of the ISO norms.

COLLAGEN MEMBRANE WITH BIOACTIVE MOLECULES TO ENHANCE BONE REGENERATION: AN *IN VITRO* STUDY

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Aim: the aim was to compare commercially available collagen membranes with the same membranes functionalized with bioactive molecules.

Methods: seventy-two collagen membranes were used. One group was functionalized with chitosan and one group with chitosan and GMBP1a peptide. Pure collagen membranes were used as controls. The following analyses were conducted: optical and two-photon microscope observations to assess optical alterations; Fourier transform infrared spectroscopy and X-ray photoemission spectroscopy to assess chemical surface characteristics; mono-axial tensile fracture to evaluate mechanical features; osteoblast proliferation, gene expression, new bone deposition, and antibacterial activity to assess biological characteristics.

Results: chemical surface analysis showed excellent integration of the bioactive molecules on the collagen membranes. Biological analysis revealed greater proliferation of human osteoblasts, greater bone matrix production, and lower bacterial proliferation on the functionalized membranes, especially with both bioactive molecules. Mechanical analysis showed no difference between hydrated pure collagen membranes and functionalized membranes, except those functionalized with only chitosan, which showed greater deformation rupture.

Conclusions: functionalized membranes reported better biological results without loss of mechanical performance. Compared with current knowledge on collagen membranes, these results demonstrated the potential advantages of chitosan and GBMP1a peptide and their possible applications in guided bone regeneration.

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BIOLOGICAL BEHAVIOR OF 3D PRINTED RESIN RESTORATIONS WITH DIFFERENT POLISHING TREATMENTS

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Aim: this study aims to evaluate the dynamics of interaction among 3D printed resin restorations presenting different surface topographies, human gingival fibroblasts and oral microorganisms.

Methods: 3D-printed samples of two commercial resins underwent different polishing treatments. Surfaces characteristics and biological behavior were analyzed with contact angle measurement, microscopical observation (SEM/AFM/Confocal), cellular viability assay, and qRT-PCR. Microbiological evaluation assessing monomicrobial or mixed species biofilm of *Streptococcus sanguinis* and *Candida albicans* was carried out in the presence and absence of an antimicrobial peptide (L18R), synthetized from the sequence of the immunoglobulin heavy J2 gene.

Results: AFM and SEM revealed differences in the surface roughness among the treatments and in samples hydrophilicity. Both the resins showed good biocompatibility, while one induced a different fibroblastic morphology. This result was also supported by the analysis of ITα-6 and IL-6 gene expression. A different amount of *S. sanguinis* and *C. albicans* total biomasses were recorded on the controls compared to the treated surfaces and in the presence/absence of L18R.

Conclusions: a different behavior of the two 3D printed resin restorations was shown on the treated surfaces as well as in the presence of the antimicrobial peptide, potentially leading to the development of novel approaches in microbial control at the transmucosal interface.

BOND STRENGTH AND ULTRAMORPHOLOGICAL EVALUATION AFTER SIMPLIFIED IMMEDIATE DENTIN SEALING

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Aim: traditionally, 3-step etch-and-rinse or 2-step self-etch adhesive systems were proposed for performing Immediate Dentin Sealing (IDS) technique. In this study, the effect of "simplified" IDS technique achieved with more user-friendly, lightly-filled universal adhesives on microtensile bond-strength (μTBS) and dentinal endogenous enzymatic activity (MMPs) was investigated.

Methods: the coronal dentin of 24 sound human molars was exposed. The following groups were formed according to the adhesive used for IDS (n = 8): 1) Clearfil Universal Bond Quick (QB); 2) Scotchbond Universal Plus (SB); 3) no IDS (CTR). A provisional restoration (Caviton) was placed. After 1 week of artificial saliva storage, CAD/CAM hybrid ceramic onlays (Katana Avencia Block) were luted using a universal resin cement (Panavia SA Cement Universal) in self-adhesive mode. The specimens were cut into 1-mm^2 thick slices and subjected to μTBS test and scanning electron microscope (SEM) analysis after 24 h (T_0) or artificial aging (10.000 thermocycles 5-55°C; T_1). *In situ* zymography was conducted on 3 additional molars per group at T_0 and T_1. Data were statistically analyzed (α = 0.05).

Results: at T_0, QB showed a significantly higher μTBS than CTR and SB ($p < 0.05$). Artificial aging negatively affected bond strength in QB and CTR, while bonding values increased in SB ($p < 0.05$). Both experimental groups demonstrated higher bond strength compared with CTR after aging ($p < 0.05$). At T_0, the IDS with the tested adhesives significantly increased the level of MMPs (QB > SB > CTR; $p < 0.05$). At T_1, only QB generated a higher gelatinolytic activity compared with CTR ($p < 0.05$).

Conclusions: the hereby proposed "simplified" IDS achieved with universal adhesive systems can have a positive impact on immediate- and aged μTBS, although it may lead to activation of MMPs within coronal dentin.

ZIRCONIUM OXYNITRATE ETCHANT IMPROVES DENTIN BONDING PROPERTIES: 5-YEAR EVALUATION

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Aim: the aim of this study was to assess, using microtensile bond strength test (μTBS) and in situ zymography, the influence of an experimental metal salt-based zirconium oxynitrate etchant [ZrO(NO3)2]-ZON, in conjunction with two simplified adhesives, on dentin bonding properties and endogenous enzymatic activity after 5 years of artificial aging.

Methods: coronal dentin surfaces (n = 8) were treated with either a traditional 37% H_3PO_4 etchant (PA) or ZON. Subsequently, a single-component etch-and-rinse adhesive (ExciteF, Ivoclar-EF) or a universal adhesive (Adhese Universal, Ivoclar-AU) was applied, followed by composite build-up and μTBS testing. Additional teeth (n = 5) were subjected to in situ zymography to evaluate dentinal gelatinolytic activity. Tests were conducted at baseline (T_0) and after a 5-year aging period (T_5). Data were statistically analyzed (α = 0.05).

Results: the dentin conditioner, adhesive system, and aging significantly influenced bond strength and enzymatic activity ($p < 0.05$). In terms of bond strength, ZON exhibited higher values compared to PA, AU outperformed EF, while bond strength decreased in all the groups after 5 years. The groups treated with ZON also showed significantly lower levels of enzymatic activity compared to PA at both T_0 and T_5 ($p < 0.05$).

Conclusions: the experimental etchant, combined with simplified adhesive systems, demonstrated higher bond strength immediately and even after 5 years of aging, potentially due to reduced collagen enzymatic degradation. The effect was particularly notable with AU, possibly due to enhanced chemical bonding.

TRABECULAR BONE-LIKE PCL/BTCP SCAFFOLD: PRELIMINARY ANALYSIS OF HUMAN OSTEOBLASTS BEHAVIOR

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Aim: Bone Tissue Engineering (BTE) holds great promise for regenerative medicine. In this project, has been investigated the behavior of human osteoblasts cultured onto 3D-Printed (3DP) trabecular bone-like shaped polycaprolactone/β-Tricalcium Phosphate scaffolds (PCL/βTCP-S), with increasing concentrations of β-TCP (0-40-70%), overcoming typical βTCP amount employed in the current literature, thus positively influencing cell/scaffold interaction and the success of bone regeneration.

Methods: PCL/βTCP-S have been produced through a solvent-free, Fused Deposition Modelling, 3DP approach and tested to evaluate human osteoblasts behavior in terms of viability (Live/Dead Assay-L/D), morphology (immunofluorescence-IF and scanning electron microscopy-SEM), differentiation and mineralization (qRT-PCR, Alizarin Red).

Results: obtained results demonstrated that osteoblasts exhibited excellent viability and proliferation rates on PCL/βTCP-S, indicating their biocompatibility and bioactivity. SEM, L/D and IF staining revealed adequate adhesion and spreading of osteoblasts throughout the surface, with the development of interconnected networks resembling native bone tissue. Furthermore, gene expression analysis and alizarin red revealed upregulation of osteogenic markers and mineralization, especially in 40% and 70% samples.

Conclusions: increasing concentrations of βTCP effectively allowed the creation of a favorable microenvironment able to elicit a positive behavior in terms of cellular adhesion, proliferation and differentiation highlighting a higher osteogenic response of composite scaffolds and their potential for BTE applications.

ACCURACY AND PRECISION OF A NEW PHOTOMETRIC JAW TRACKING SYSTEM ON THE FRONTAL PLANE

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Aim: to assess accuracy (trueness and precision) of a new photometric Jaw Tracking System (JTS) in recording linear movements on the frontal plane at different recording distances.

Methods: a mandibular plaster cast of a patient was placed on a simulation machine able to move linearly on two spatial axis. The CYCLOPS Mandyfork (ITAKA Way Med) was adapted to the plaster cast while the Upper Positioner was fixed to the simulation machine. Linear movements from 20 to 40 mm on y-axis were performed in quintuplicate at 5 different distance positions (from 380 to 420 mm) of the plaster cast from the scanner. The movements were compared with those retrieved by a laser vibrometer for accuracy analysis. Data were statistically processed ($\alpha = 0.5$) using ANOVA test.

Results: no statistically significant differences were highlighted between CYCLOPS and laser vibrometer measurements on y- and z-axis ($p = 0.5$). The best-performing conditions was with an amplitude of 30 mm at 420 mm, reporting an overall accuracy of 0.010 mm. Amplitude of movements and distance position did not affect the accuracy of the instrument, trueness and precision values above 99% have generally been reported.

Conclusions: cyclops demonstrated to be an accurate system. Trueness is more sensitive to vertical amplitude parameter variation than the different position of the scanner during the recordings. For good clinical practice, it would be better to position the scanner as close as possible to the patient according to the subject degree of opening, in order to facilitate the detection of markers during movements.

MULTIPLE ACIDIC SOLUTION FOR ETCHING ZIRCONIA RESTORATIONS

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Aim: recently, a multiacid solution (HF, HCl, H_2SO_4, HNO_3, H_3PO_4, Zircos–E,Bio Den Co) has been proposed as an alternative pretreatment option of zirconia restorations compared to the gold standard sandblasting. The influence of zirconia pretreatments and cementation techniques on μshear bond strength (μSBS) and surface properties at baseline and after aging was investigated.

Methods: zirconia blocks (Biodynamics) were cut into 12 x 4 x 4 mm slices and divided in groups according to pretreatment: SB: sintering, sand-blasting (Al_2O_3 50 μm, 2 bar), or ZE: as in SB + etched with Zircos–E for 30 min. Resin composite cylinders (n = 15 per group) were cemented on the zirconia with a universal cement (Panavia Universal, Kuraray) with/without a universal adhesive (Clearfil Universal Bond Quick, Kuraray) and subjected to μSBS test after 24h (T_0) or after thermocycling (10.000, 5-55°C cycles; T_1). Specimens' morphologies were observed under SEM. Data were statistically analyzed ($p < 0.05$).

Results: pretreatment, cementation, aging, and their interactions influenced μSBS significantly ($p < 0.05$). ZE yielded higher bond strength compared to SB. While in the SB the use of adhesive in the cementation procedure increased bond strength, there were no differences between the cementation strategies in the ZE group ($p > 0.05$).

Conclusions: although zirconia is generally considered a non-etchable material, it seems that the multiacid solution Zircos-E prior to cementation ensures a high and durable bond strength, even in case of a simplified cementation procedure.

BIOLOGICAL AND MECHANICAL CHARACTERIZATION OF A NEW ANTIBACTERIAL NANO-FILLED COMPOSITE

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Aim: biological, morphological, and mechanical characterization of a new silver- based dental composite filler material for the treatment of vulnerable and fragile patients.

Methods: silica or hydroxyapatite supported silver nanoparticles were prepared. Composites were functionalized with a coupling silane agent and mixed to the organic matrix fillers. Tetric PowerFill IVA composite (Ivoclar) was used as control. Cytotoxicity from acute exposure (24 h, 48 h and 72 h) was evaluated on human oral fibroblasts with MTT assay, cells morphology with SEM analysis, and tensile and compressive (45° and bending) tests with LLOYD Instruments LR30 K dynamometer (load cell of 30 KN, and 1 mm/min speed).

Results: silver- based dental composite filler material showed high cytotoxicity at 24 h and 48 h ($p < 0.05$), and at 72 h ($p < 0.01$). SEM analysis confirmed morphological alterations, showing important morphological alterations: altered cell shapes, raised edges and absence of filopodia and lamellipodia. From the analysis of the maximum load and stiffness, considering the average values, the specimen exhibited highest values, with good adhesion and mechanical characteristics. During compressive tests some of the specimens fractured.

Conclusions: the results contribute to the advancement of knowledge in the field of dental materials to improve dental care in high-risk patients by considering the addition of nanoparticles with antibacterial function. Future studies are needed to improve biological and mechanical properties, considering the evaluation of genotoxicity, and microbiological characteristics.

3D POROUS GRAPHENE AEROGEL: A NEW APPLICATION IN DENTISTRY

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Aim: the aim of this *in vitro* study is to use a graphene-based aerogel compound, which is designed to highly adsorb aqueous substances, and which could be useful in clinical procedures improving the quality of fluid intake and filtration of microorganisms.

Methods: a highly porous graphene-based aerogel compound has been developed in the CNIS of Sapienza University of Rome, using the solution casting method of production. A parallelepiped insert measuring 4 mm in height with a base of 3 x 2 mm has been obtained from the original graphene sample and then inserted into the cap of a plastic suction device. To simulate the suction system of dental units, the saliva suction device has been connected to a mini-vacuum pump. Several suctions tests have been performed, both with the normal suction device and with the modified one, at a pressure of 200 mbar. Each test was accurately recorded at high magnification.

Results: both the intake systems are well working. However, for the classic suction system, the saliva suction device must be completely inserted deeply into the liquid to perform its complete suction. On the contrary, for the suction device with graphene insert, the same level of suction is obtained keeping the cap just in contact with the surface of the liquid.

Conclusions: it is possible to improve the quality of the suction by adding a small parallelepiped of aerogel graphene in the cap of a saliva suction device. The hydrophilic graphene insert can make clinical procedures easier, improving the visibility of the operating field and reducing the risk of cross-contamination.

MECHANICAL PROPERTIES CHARACTERIZATION OF 3D PRINTED PCL SCAFFOLDS FOR BONE REGENERATION

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Aim: Polycaprolactone (PCL) has recently been adopted as biomaterial thanks to its biological and mechanical properties which emerged to be suitable for bone tissue regeneration. The present study focuses on the analysis of the mechanical performance of 3D printed PCL scaffolds.

Methods: the PCL scaffolds were printed to obtain a porous cubic structure. Post-production modifications were performed to improve bioactivity and cell adhesion obtaining 4 different groups: non treated PCL, PCL activated with air-plasma, PCL activated with air-plasma and soaked in nHAp, lastly PCL activated with air-plasma, soaked in nHAp and sterilized with UV rays. The groups were tested using a universal testing machine to evaluate both compression strength and elastic modulus. Wettability was also investigated by measuring the contact angle of the differently treated scaffolds. This was done to observe if the superficial treatments improved the hydrophilicity of PCL in order to promote cell adhesion and proliferation. The morphology of the scaffolds was investigated by means of Scanning Electron Microscope (SEM).

Results: the results highlighted statistically significant variations in the elastic modulus between the groups treated with nHAp compared to the others. The elastic modulus range for PCL is 70-90 MPa, therefore compatible with trabecular bone. PCL wettability was improved thanks to the air-plasma treatment.

Conclusions: within this study limitations, PCL scaffolds were shown to be a promising biomaterial for bone regeneration purposes.

EVIDENCE OF MICROPLASTIC DETACHMENT ON ORTHODONTIC ALIGNERS: A SPECTROSCOPIC STUDY

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Aim: this *in vitro* study evaluates the potential detachment of microplastics (MPs) by clear orthodontic aligners due to mechanical friction simulated by a 7-days protocol in artificial saliva.

Methods: clear orthodontic aligners from different manufacturers were evaluated: Alleo (AL), Flexi Ligner (FL), F22 Aligner (F22), Invisalign (INV), Lineo (LIN), ArcAngel (AR), Ortobel Aligner (OR), SureSmile (SS), Spark (SP), and Graphi (GP). For each group, two aligners were immersed in artificial saliva and stirred for 5 hours/day, mimicking the physiological teeth mechanical friction. After 7 days, the artificial saliva was filtered under vacuum through 1.6 m pore-size filter membranes (Whatman GF/A). Filters were analyzed by Raman Microspectroscopy (RMS) and Scanning Electron Microscopy (SEM), respectively to chemically identify the polymeric matrix and to measure the number, size and shape of MPs. One-way ANOVA, followed by Tukey's multiple comparisons test, was performed. Statistical significance was set at $p < 0.05$.

Results: RMS spectra revealed that F22 and INV were made by polyurethane, while AL, FL, LIN, AR, and OR by polyethylene terephthalate. By preliminary SEM investigation, the highest number of MPs was found in ARC, SP, and GP, while the lowest one in INV ($p < 0.05$). As regards MPs' size, statistically significant differences were found among groups ($p < 0.05$), with most MPs ranging from 5 to 20 µm, while other ones reaching 100-200 µm. A spherical shape was observed in AL, FL, INV, OR and GP, while a fiber one in F22, LIN, ARC, SS and SP.

Conclusions: this study highlighted for the first time the detachment of MPs from clear aligners due to mechanical friction. This evidence may represent a great concern in clinical practice, since it could impact human general health.

PHYSICAL PROPERTIES IN CAD/CAM COMPLETE DENTURE RESINS

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Aim: the shift toward using Computer-Aided Design and Computer-Aided Manufacturing (CAD/CAM) systems in dentistry is increasing, replacing traditional laboratory techniques. Despite this trend, there remains a scarcity of studies that compare different CAD/CAM materials. This research was conducted to evaluate and compare the color stability and hardness of gingival and tooth-colored milled and 3D-printed acrylic resins.

Methods: this study involved four types of CAD/CAM materials: 3D-printed in pink shade (PP), milled polymethylmethacrylate (PMMA) in pink shade (MP), 3D-printed in tooth shade (PT), and milled PMMA in tooth shade (MT), with six samples each. Disc-shaped samples (15 mm x 2 mm) were used for hardness testing and bar-shaped samples (65 mm x 10 mm x 2.5 mm) for color stability assessments, all of which were polished. Hardness was tested using the Vickers hardness test on a microhardness tester. Statistical analysis was conducted using one-way ANOVA and Tukey's post hoc tests.

Results: the milled PMMA samples exhibited superior color stability compared to the 3D-printed resins. Both pink and tooth shade milled samples showed similar color stability, while 3D-printed tooth shade samples displayed better color stability than their pink counterparts. In terms of hardness, milled tooth shade PMMA ranked highest, followed by 3D-printed tooth shade.

Conclusions: milled PMMA offers better color stability than 3D-printed resins. Additionally, both milled and 3D-printed tooth shade resins are harder than pink shade equivalents.

RETENTION STRENGTH OF 4 CEMENTS DESIGNED FOR TITANIUM/ ZIRCONIA IMPLANT RESTORATIONS

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Aim: several resin cements dedicated to the cementation of translucent materials to metal abutments have been recently introduced in implant prosthodontics; they could have adhesives and/or primers to be applied separately or be of the self-adhesive type. Dull white colored, the optical properties of these materials are tuned to obtain a strong masking effect over dark substrates like metals.

Methods: 4 cement groups (n = 10): A) Multilink Abutment (Ivoclar); B) OverCEM Ti-Abutment (Overfibers); C) Bifix Hybrid Abutment (VOCO); D) Panavia V5 Opaque (Kuraray); they were used to bond a purposely designed zirconia crown (Katana STML, Kuraray) to a titanium abutment screwed to an implant (Premium 4.25 mm, Sweden&Martina). Both abutment and ceramic were air-abraded with 50 m Al_2O_3. Once cemented following the manufacturer instructions, the specimens were stored in water at 37°C for 24hrs then subjected to a pull-out test (Instron 2530).

Results: significant differences were found between groups A-B and A-D (Kruskal-Wallis and Dunn's test, α = 0.0083). OverCEM Ti-Abutment showed the highest retention strength with 710.3±167N followed by V5 487.4±99N, Bifix 432.6±167N, and Multilink 243.9±92N. Stereomicroscope analysis revealed ZrO_2/Ti failure patterns %: A: 50/50; B: 50/50; C: 50/50; D:10/90.

Conclusions: retention strength showed a high SD in all groups suggesting a certain sensibility to the application technique. OverCEM Ti Abutment, a dual-curing material that requires a heating treatment at 50°C after light polymerization, gave the highest forces: a remarkable result for a cement that do not require any primer. V5 and Bifix showed adequate retention strength, although obtained with the use of a ceramic primer. Multilink Abutment do not require neither light curing nor primers, it was simple to use but its performances were fair.

BACTERIAL ADHESION TO TURNED AND NANOSTRUCTURED TITANIUM IMPLANT SURFACES

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Aim: aim of the present study was to investigate the bacterial adhesion to turned and nanostructured titanium (Ti) implant surfaces.

Methods: turned and oxidized samples of commercially pure Ti were topographically analyzed by 3D profilometry. Bacterial cultures (*Streptococcus sanguinis*) were *in vitro* seeded and, after two hours, adherent bacteria were quantified by Colony-Forming Unit (CFU) counting. Ti samples were also exposed to the oral environment of six periodontally healthy volunteers and, after 12h, the deposed biofilm was evaluated by CFU counting. Inter-group differences were tested by the Mann–Whitney U-Test ($\alpha = 0.05$).

Results: 3D profilometry analysis showed a grooved surface for turned surfaces and a more complex micro- and nano-scaled texture for nanostructured samples. *In vitro* tests showed a lower bacterial adhesion for nanostructured than turned surfaces at two hours ($p = 0.04$). Non-significant differences emerged from 12h *ex-vivo* tests between the two types of surfaces.

Conclusions: nanostructured surfaces showed a lower early bacterial adhesion *in vitro* compared to turned surfaces, although such a difference was not confirmed by the 12 h *ex vivo* test. Implant surface characteristics could exert possible implications on the health of peri-implant tissues over time and their choice should be carefully made based on solid scientific evidence.